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RESEARCH PAPER

Investigation and optimization of the purification process of anthocyanins from *Aronia melanocarpa* (L.) Nieuwl.

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Abstract

In this study, an ultrasound-assisted deep eutectic solvent was used to extract anthocyanins from *Aronia melanocarpa* (L.) Nieuwl. 1,2-Propanediol, glycerol, lactic acid, citric acid and glucose were used as hydrogen bond donors (HBS) and acceptors. A choline chloride (HBA) eutectic reaction was performed according to the extraction rate of anthocyanins to characterize the deep eutectic solvent. The results revealed that the optimal solvent raw materials were choline chloride and glycerol, and the optimum extraction conditions were: ultrasonic treatment power of 165 W, time of 20 min, and solvent moisture content of 40%. Under these conditions, the extraction amount of anthocyanin was 42.541 ± 0.15 mg/g, close to the prediction of the regression model (42.555 mg/g). The model analysis revealed that ultrasonic power demonstrated the most significant impact on extraction yield ($F = 572.095$, $p < 0.0001$), followed by ultrasonic duration and moisture content. During purification, UV scanning identified 540 nm as the optimal absorption wavelength for anthocyanins. Comparative studies of three macroporous resins (X-5, AB-8, polyacrylamide) showed X-5 resin exhibited superior performance in static adsorption, achieving equilibrium within 1 hour with high desorption efficiency. In dynamic elution, X-5 reached peak absorbance at 10 mL elution volume, outperforming polyacrylamide. Consequently, X-5 resin was selected as the purification medium with 80% ethanol eluent, which maximized anthocyanin desorption stability. This study establishes an efficient and environmentally friendly extraction-purification process for *Aronia melanocarpa* anthocyanins, providing robust theoretical foundations and technical support for industrial development with significant application potential.

Keywords

Anthocyanin, *Aronia melanocarpa*, deep eutectic solvent, purification

Introduction

Aronia melanocarpa, commonly known as black chokeberry, is a perennial deciduous shrub of the genus *Aronia* in the Rosaceae family. Native to North America, it is naturally distributed between the northeastern part of the Great Lakes region and the upper mountain swamps of the Appalachian Mountains (Yu 2020). *Aronia melanocarpa* is rich in various active substances and possesses extremely high nutritional and medical value. Fruits are rich in various organic acids, polyphenolic compounds, and polysaccharide substances (Hu et al. 2020; Yu et al. 2020; Wang et al. 2021a). Among them, the content of phenolic compounds in *Aronia melanocarpa* far exceeds that of most plants, typically consisting of monomeric polyphenols such as phenolic acids, flavonoids, and anthocyanins, and polymeric polyphenols mainly represented by proanthocyanidins, which are located in the epidermal layer of the pericarp (Jia et al. 2020; Wang et al. 2020b). Among these compounds, the most abundant are anthocyanin compounds, accounting for approximately 20% of the

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total (Ho et al. 2014; Sun et al. 2017; Li et al. 2019; Zhao et al. 2021), and they have multiple biological activities, such as antioxidation and anti-inflammatory activities, indicating broad application prospects in the fields of food and medicine. During the extraction process, traditional extraction methods suffer from drawbacks such as low efficiency and significant pollution, restricting the efficient utilization of resources. Deep eutectic solvents (DESs), which are primarily composed of hydrogen bond donors and acceptors, form a liquid with a melting point lower than their own through hydrogen bonding when uniformly mixed at a certain temperature. As novel sustainable solvents, DESs have gradually garnered attention in recent years because of their distinctive physicochemical properties and advantages (Shang et al. 2017; Wang et al. 2020a; Du et al. 2021), such as adjustable solution viscosity, low volatility, high solubility, low toxicity, nonflammability, and ease of preparation. In recent years, DESs have gradually replaced common organic solvents and ionic liquids, emerging as one of the most favored extraction solvents and offering a new direction for the extraction of anthocyanins from *Aronia melanocarpa*. To facilitate the green development and efficient utilization of *Aronia melanocarpa*, this research employed multiple deep eutectic solvents in conjunction with ultrasonic treatment-assisted extraction to extract anthocyanins from *Aronia melanocarpa*. The influences of factors such as the type of DES, water content, ultrasonic treatment time, and ultrasonic treatment power on the extraction were systematically investigated, and the extraction process was optimized using the response surface methodology. The objectives of this study are to establish an accurate extraction model, increase the extraction rate of anthocyanins, explore the purification process of anthocyanins, screen suitable macroporous resins and elution reagents, determine the optimal purification conditions, provide a solid theoretical foundation and technical support for the industrial production of anthocyanins from *Aronia melanocarpa*, and promote their wide application in related industries.

Materials and methods

Materials and instruments and equipment

The fruits of *Aronia melanocarpa*: Cultivar Fukangyuan 1 of *Aronia melanocarpa* in Zhaodong City, Heilongjiang Province were used in this study. The fruits were harvested after reaching maturity, which occurred 103 days after flowering, typically between late August and early September. This cultivar grows in loose, fertile, well-drained soil. The planting period was 4 years, with fertilization using farmyard manure and regular irrigation. The growing environment is characterized by a warm and humid climate, with an average annual temperature of approximately 10 °C and annual precipitation ranging from 600 to 800 mm.

Crusher: Maierkang Environmental Protection Technology Co., Ltd.; Spectrophotometer: Beijing Puxi General Instrument Co., Ltd.; Electronic balance: Mettler-Toledo Instrument Co., Ltd.; High-speed centrifuge: Anxin Experimental Instrument Co., Ltd.; EMS-11A constant-temperature bidirectional stirrer: Tianjin Ouno Instrument and Meter Co., Ltd.; High-temperature water bath: Shanghai YIHENG Scientific Instrument Co., Ltd.; and Constant-temperature drying oven: Shanghai Boxun Industrial Co., Ltd.

Experimental methods

Raw material pretreatment

Fresh black fruits of *Aronia melanocarpa* were selected and washed, and bad ones were removed. A certain amount of ascorbic acid was added for color protection, followed by juicing. Cellulase and pectinase with a mass fraction of 0.5% were added to the crushed fruit pulp to facilitate the precipitation of the contents (Wang et al. 2021b).

Determination of anthocyanin content

The anthocyanin content was determined via the pH differential method: 1.0 mL of the anthocyanin extract was added, and the volume was increased to 5 mL with distilled water. Two aliquots of the diluted sample mixture, 0.5 mL each, were transferred, and the volume was increased to 5 mL with pH 1.0 and pH 4.5 buffer solutions. The samples were incubated in a 40 °C water bath for equilibration for 30 minutes. Distilled water was used instead of the sample as the blank control. The absorbance was measured at 510 nm and 700 nm. Once all the absorbance measurements were completed, calculations were performed.

Anthocyanin extraction process

After the raw material pomace was subjected to a cleaning treatment, the black fruit of *Aronia melanocarpa* pomace was passed through a sieve, and 0.5 g of powder was weighed and homogeneously mixed with a certain quantity of DES solution. Once completely mixed, it was placed in an ultrasonic processor for a certain period of time. Upon completion of the treatment, a certain amount of distilled water was added to terminate the reaction. The resulting mixture was centrifuged at 8000 rpm/min for 10 minutes, after which the supernatant was extracted. The residue was subjected to a secondary extraction and then centrifuged together. The outcomes of the two extractions were combined to obtain the anthocyanin extract (Shi 2020).

Single-factor experiments for extraction process optimization

With anthocyanin content serving as the dependent variable, the DES type, DES moisture content, ultrasonic

treatment time, and ultrasonic treatment power were selected as independent variables, and experiments were carried out under the premise that the other variables were single variables.

Determination of DES types

In this study, choline chloride was selected as the hydrogen bond acceptor, and five types of organic substances were chosen as the hydrogen bond donors. The ratios of the mixture with choline chloride were determined according to the structural differences in the hydrogen bond donors, as specified in Table 1.

Table 1. Types of eutectic solvents.

Number	Composition of deep eutectic solvents		
	HBA	HBS	Mole ratio
DES-1	Choline chloride	1,2-Propanediol	1:2
DES-2	Choline chloride	Glycerol	1:2
DES-3	Choline chloride	Lactic acid	1:1
DES-4	Choline chloride	Citric acid	1:4
DES-5	Choline chloride	Glucose	1:1

In accordance with the ratios in Table 1, the raw materials were mixed with the different numbered DESs. The mixed raw materials were heated to 80 °C and continuously stirred until the solution was clear. After the solution cooled, it was stored for later use.

When the DES moisture content was 30%, the ultrasonic treatment time was 25 minutes, and the ultrasonic treatment power was 200 W, the extraction quantity of anthocyanins in different DES solutions was calculated. The optimal DES solution was selected as the extraction solvent for the next step of the experiment.

Influence of moisture content on the extraction amount

When the ultrasonic treatment time was 25 minutes and the ultrasonic treatment power was 200 W, moisture contents of 10%, 20%, 30%, 40%, and 50% were used. The final moisture content was determined in accordance with the anthocyanin extraction quantity.

Influence of ultrasonic treatment time on the extraction amount

Under the conditions where the moisture content was 30% and the ultrasonic treatment power was 200 W, the ultrasonic treatment times were set at 10 min, 20 min, 30 min, 40 min, and 50 min. The ultrasonic treatment time was determined in accordance with the anthocyanin extraction quantity.

Influence of ultrasonic treatment power on the extraction amount

When the ultrasonic treatment time was 25 min and the moisture content was 30%, the ultrasonic treatment powers were set at 100 W, 150 W, 200 W, 250 W, and 300 W, respectively. The ultrasonic treatment power was determined in accordance with the anthocyanin extraction quantity.

Optimization conditions of the extraction process response surface

On the basis of the single-factor experiments, the ultrasonic treatment power (A), ultrasonic treatment time (B), and moisture content (C) were taken as the independent variables for investigation. Three relatively superior levels were identified from the five levels in the single-factor experiments. With the anthocyanin extraction amount (Y) as the indicator, the Box–Behnken module of the Design–Expert software was used to design a total of 17 response surface experiments with three factors and three levels.

Technology for the purification of anthocyanins from *Aronia melanocarpa*

Screening of macroporous resins

The main factors that affect the adsorption performance of macroporous resins include pore size, particle size, and polarity. Considering the types and contents of the substances to be separated, as well as the cost of the stationary phase, three types of macroporous resins, namely, polyacrylamide, AB-8, and X-5, were selected for comparative experiments in this test to select the optimal medium.

Determination of the maximum absorption peak

After the extraction process was optimized, the anthocyanins of *Aronia melanocarpa* were subjected to ultraviolet spectral scanning within the wavelength range of 150–800 nm via an ultraviolet spectrophotometer. The maximum absorption peak of the anthocyanins was determined on the basis of the displayed maximum wavelength.

Upper column conditions

The volume of the chromatographic column was 4 cm × 100 cm; the diameter-to-height ratio was 1:10; the sample volume was 1/5 of the column volume; the sample loading concentration was 2 mg/mL; and the flow rate was 1 mL/min. Elution ceased when the absorbance of the eluted product reached 1/5 of the maximum absorbance during the elution process.

Comparison of the static adsorption capacity of macroporous resins

First, 10.0 g of macroporous resin was accurately weighed. After pretreatment, the sample was added, and thermostatic oscillation was conducted at 25 °C and 100 rpm/min for 2.5 hours. Samples were taken every 0.5 hours. Absorbance analysis was carried out on the extracted samples, and the adsorption rate was calculated accordingly.

Comparison of the static desorption capacity of macroporous resins

Following the completion of the static adsorption experiment described in the upper column conditions, the saturated resin containing the adsorbed material was trans-

ferred to a new conical flask, and 50 mL of 60% ethanol was added as the desorption solvent. The flask was then placed in a thermostatic shaker for desorption at 25 °C with a shaking speed of 100 rpm/min for 2.5 hours. Samples were collected every 0.5 hours for absorbance analysis, and the desorption efficiency was calculated on the basis of these measurements.

Comparison of the dynamic elution capacity of macroporous resins

Dynamic elution was conducted using ethanol at a specified concentration. The crude extract was first wet-packed onto the column. After loading, the column was rinsed with two column volumes of distilled water, followed by elution with ethanol. Absorbance measurements were taken for each 5 mL fraction collected until the maximum absorption peak was reached. The elution profile was then plotted, and the anthocyanin yield was calculated on the basis of these data.

Selection of elution reagents

Following the completion of static adsorption and desorption experiments and a comparison of dynamic elution performance, a single-factor experiment was conducted to select the purification mobile phase, with the anthocyanin purification rate used as the reference criterion.

The pretreated X-5 and polyacrylamide resins were accurately weighed for column packing. Ethanol solutions ranging from 40% to 90% were prepared as the mobile phase, with the volume of each solution being twice that of the column. The relationship between anthocyanin desorption and mobile phase composition was analyzed through desorption rate analysis at different ethanol concentrations.

Statistical analysis

All data were expressed as mean ± standard deviation cal culated from the results of 3 or more experiments. Results were considered significant at $p < 0.05$ using one-way ANOVA. Data analysis and processing were performed using software tools, including Origin 2022b, SPSS 21.0, and Design-Expert 8.0.

Results and analysis

Results of single-factor experiments

Screening results of DES solutions

In this study, five deep eutectic solvents (DES-1 to DES-5) were designed for anthocyanin extraction. As shown in Fig. 1A, DES-2, composed of glycerol and choline chloride, demonstrated a significantly higher extraction yield than the other DES formulations did. The extraction efficiency was consistently stable across multiple trials, with the highest recorded yield reaching 7.39 mg/g. This performance surpasses that of conventional ultrasonic and organic solvent extraction methods. Therefore, we selected DES-2 to assist in micro-ultrasonic treatment for subsequent experiments.

Influence of moisture content on the extraction amount of anthocyanins

Given the relatively high viscosity of the DES solution, a specific amount of distilled water was added as a reaction medium. This series of single-factor experiments investigated the impact of varying the mass fraction of distilled water from 10% to 50% on the anthocyanin extraction yield.

The results are presented in Fig. 1B. When the mass fraction of distilled water was increased to 40%, a significant increase in the extraction yield was observed, reaching 46.172 mg/g. This enhancement is attributed primarily to the ability of an appropriate amount of water to reduce the viscosity of the DES solution and modulate its polarity, thereby facilitating the precipitation of anthocyanins. However, excessive water content may lead to hydrogen bond disruption, potentially affecting the extraction efficiency. Given that the anthocyanin content at 30% water content was significantly greater than that at other levels, response surface methodology (RSM) experiments were conducted at 35%, 40%, and 45% water contents.

Influence of ultrasonic treatment time on the extraction amount of anthocyanins

The results are presented in Fig. 1C. Within the ultrasonic treatment duration of 10 to 20 min, the extraction yield rapidly increased, peaking at 44.002 mg/g, followed by a gradual decrease. After 30 minutes, the anthocyanin content did not significantly differ, indicating that a 20-minute treatment is sufficient for the efficient extraction of anthocyanins from the pomace. The principle of ultrasonic treatment involves accelerating molecular motion and friction, leading to an increase in temperature. Prolonged ultrasonic exposure can cause thermal degradation or decomposition of the target compounds, thereby adversely affecting the extraction efficiency. Therefore, three levels—15 minutes, 20 minutes, and 25 minutes—were selected for the response surface methodology (RSM) experiments.

Influence of ultrasonic treatment power on the extraction amount of anthocyanins

The results are presented in Fig. 1D. The extraction yield of anthocyanins initially increased and subsequently decreased with increasing ultrasonic treatment power, reaching a peak at approximately 200 W, with an extraction yield of 41.58 mg/g. When the power exceeded 200 W, the extraction yield stabilized but remained lower than that observed at 200 W. This behavior is analogous to the effect of increasing the ultrasonic treatment duration, where within a certain range, higher power facilitates the permeation and dissolution of raw materials. However, owing to the poor stability and susceptibility to decomposition of anthocyanin compounds, it is crucial

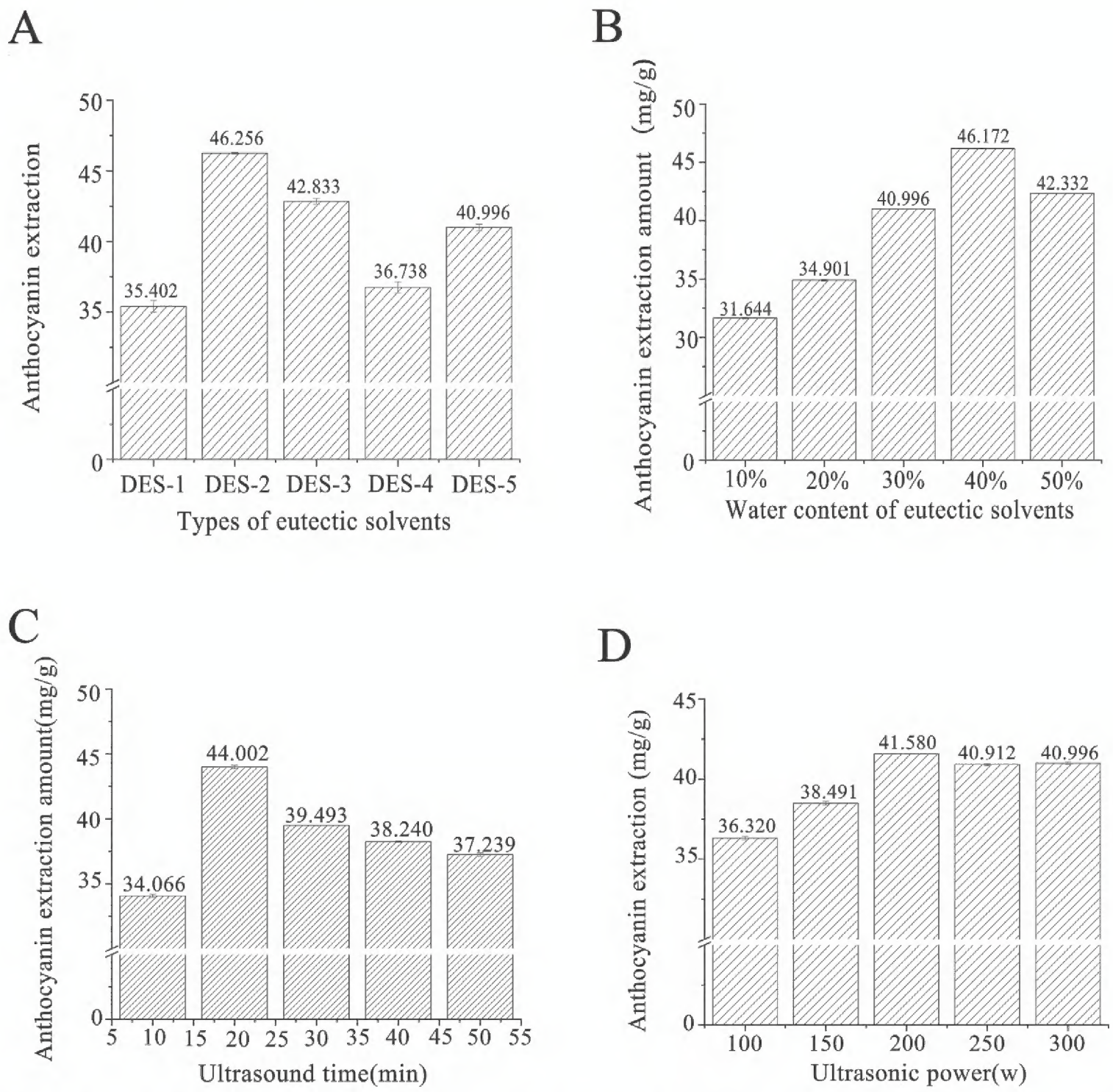

Figure 1. A. Screening results of DESs; **B.** Effect of distilled water content on the extraction rate; **C.** Effect of ultrasonic treatment time on the extraction rate; **D.** Effect of ultrasonic treatment power on the extraction rate.

to strictly control the reaction power during experimentation. Therefore, three power levels—150 W, 200 W, and 250 W—were selected for the response surface methodology (RSM) experiments.

Response surface optimization results and analysis

Determination of the response surface model

On the basis of the results of the single-factor experiments, ultrasonic treatment power (A), ultrasonic treatment duration (B), and moisture content (C) were selected as the independent variables for further investigation. Three levels for each factor were determined from the single-factor experiments to conduct a response surface methodology (RSM) experiment with three factors and three levels, resulting in a total of 17 experimental runs. The specific details are summarized in Table 2.

The response surface experimental data were analyzed using multiple regression to derive the quadratic multiple regression model equation for the anthocyanin extraction yield (Y) as a function of ultrasonic treatment power (A), ultrasonic treatment duration (B), and moisture content (C). The analysis of variance (ANOVA) results for this model are summarized in Table 3.

Table 2. Response surface experimental factors and results.

Sequence Number	Experimental factors			Extraction yield (Y), mg/g
	(A) Ultrasonic power, W	(B) Ultrasound time, min	(C) Moisture content, %	
1	45	20	250	38.06
2	40	20	200	41.95
3	45	20	150	37.37
4	45	25	200	37.86
5	35	20	150	40.44
6	40	15	150	38.27
7	40	20	200	42.07
8	35	25	200	40.32
9	40	20	200	42.35
10	40	15	250	38.87
11	40	25	150	37.99
12	40	20	200	41.89
13	40	20	200	42.21
14	35	15	200	40.91
15	40	25	250	37.94
16	35	20	250	40.45
17	45	15	200	38.32

$$Y = 42.09 - 1.31A - 0.28B + 0.16C + 0.032AB + 0.17AC - 0.16BC - 0.96A^2 - 1.78B^2 - 2.05C^2$$

Table 3. Analysis of variance of the response surface fitting regression equation.

Source of Variance	Sum of Squares	Degree of Freedom	Mean Square Deviation	F - Value	p	Significance
Model	53.421	9	5.936	245.938	<0.0001	**
A	13.808	1	13.808	572.095	<0.0001	**
B	0.638	1	0.638	26.453	0.0013	**
C	0.195	1	0.195	8.093	0.0249	*
AB	0.004	1	0.004	0.175	0.6882	
AC	0.116	1	0.116	4.790	0.0648	
BC	0.106	1	0.106	4.376	0.0748	
A^2	3.917	1	3.917	162.291	<0.0001	**
B^2	13.296	1	13.296	550.889	<0.0001	**
C^2	17.686	1	17.686	732.799	<0.0001	**
Residual	0.169	7	0.024			
Lack-of-fit term	0.027	3	0.009	0.254	0.8554	Not significant
Pure error	0.142	4	0.035			
Total	53.590	16				

Note: * Indicates a significant difference, $p < 0.05$; ** indicates an extremely significant difference, $p < 0.01$; pre $R^2 = 0.9878$.

As indicated in Table 3, the p-value of the model is less than 0.01, confirming its statistical significance at the 99% confidence level. Conversely, the lack-of-fit term has a p-value of 0.8554 ($p > 0.05$), indicating that this term is not statistically significant. These findings suggest that the model adequately captures the influence of ultrasonic treatment power, ultrasonic treatment time, and moisture content on anthocyanin yield during the extraction process. Furthermore, the close agreement between the predicted and observed values supports the reliability of this model for predicting anthocyanin extraction outcomes.

The coefficient of determination (R^2) for the model is 0.9968, and the adjusted coefficient of determination (Adj R^2) is 0.9928, which exceeds the threshold of 0.8. Additionally, the coefficient of variation (CV%) is 0.39. These metrics collectively indicate that the model has excellent fitting performance and is suitable for analyzing and predicting the anthocyanin extraction process. The signal-to-noise ratio (SNR) of 39.844 further corroborates the robustness and reliability of the model.

In this study, the first-order factors A (ultrasonic treatment power) and B (ultrasonic treatment time), as well as the second-order factors A^2, B^2, and C^2, had highly significant effects on the outcome ($p < 0.01$). The first-order factor C (moisture content) had a significant effect on the results ($p < 0.05$). On the basis of the F values, the relative influence of these three factors on the anthocyanin extraction yield is ranked as follows: A (ultrasonic treatment power) > B (ultrasonic treatment time) > C (moisture content).

Interaction analysis of factors in the response surface

The response surface curves were generated on the basis of the interactions among the various factors, as illustrated in Fig. 2A–C).

On the basis of Fig. 2A–C, the interaction effects among the three factors on the anthocyanin extraction yield can be analyzed through the shapes of the response surface plots and contour plots. The highest point on the response surface within the selected range of the two factors represents the theoretical maximum anthocyanin extraction yield.

The peak extraction yield was achieved when the ultrasonic treatment power was within the range of 150– to 175 W and the ultrasonic treatment time was between 19 and 21 minutes. The influence of ultrasonic treatment power on the extraction rate was more pronounced than that of ultrasonic treatment time. Specifically, the maximum extraction yield was observed at an ultrasonic treatment power of approximately 165 W and an ultrasonic treatment time of approximately 19 minutes. However, the interaction effect between these two factors was only moderately significant.

The peak extraction yield was achieved when the ultrasonic treatment power was within the range of 150 to 175 W and the moisture content was approximately 40%. The influence of ultrasonic treatment power on the extraction rate was more pronounced than that of the moisture content. Specifically, the maximum extraction yield was observed at a liquid-to-solid ratio of approximately 33 mL/g and a moisture content of approximately 40%. Additionally, the interaction between these two factors was statistically significant.

The peak extraction yield was achieved when the ultrasonic treatment time was within the range of 19– to 21 min and the moisture content was approximately 40%. The influence of ultrasonic treatment time on the extraction rate was more pronounced than that of the moisture content. Specifically, the maximum extraction yield was observed at an ultrasonic treatment time of approximately 20 minutes and a moisture content of approximately 40%. The interaction between these two factors was statistically significant. These findings are consistent with the results of the variance analysis.

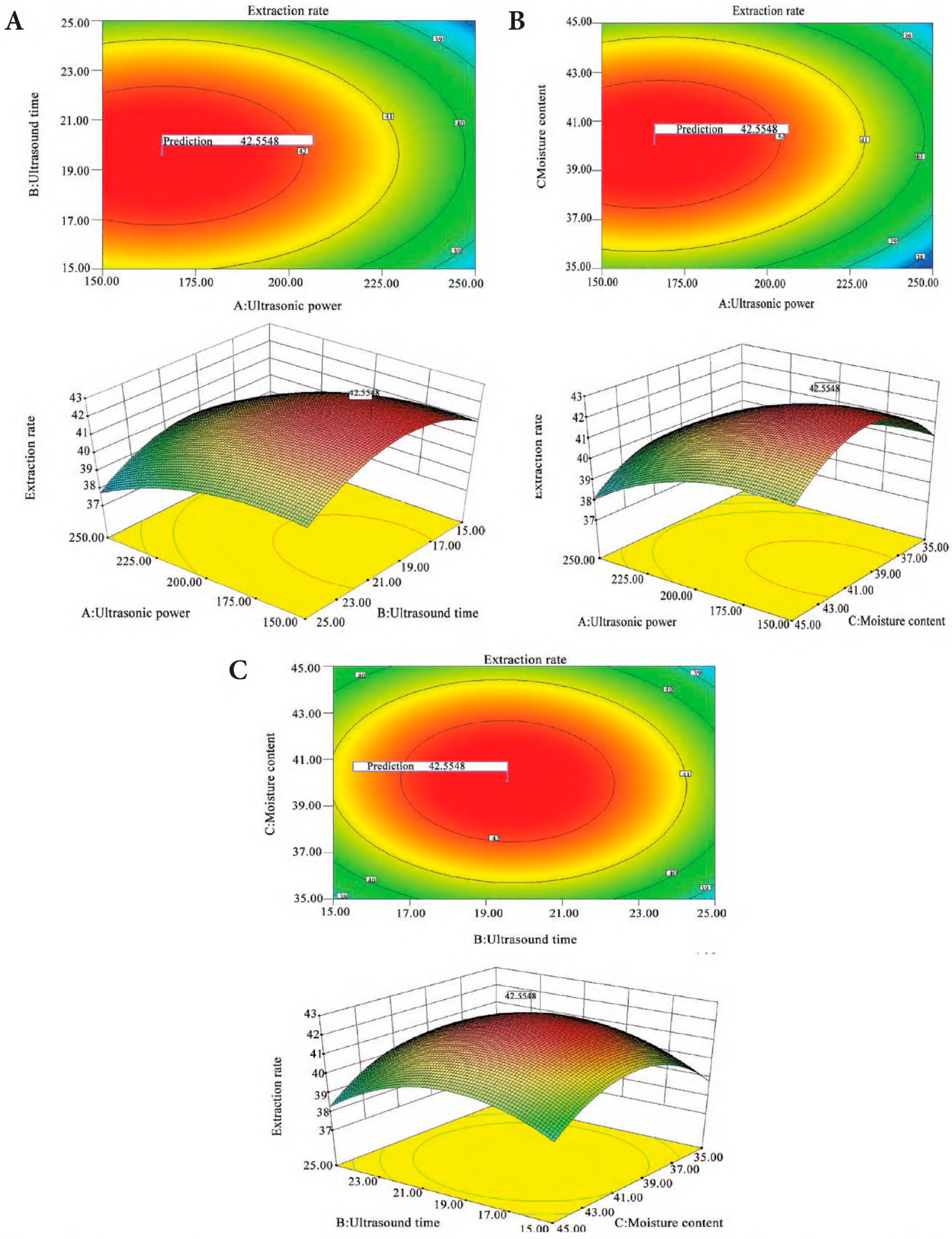

Figure 2. A. Response surface and contour plots of the interaction effects of ultrasonic treatment time and ultrasonic treatment power on anthocyanin extraction; **B.** Response surface and contour plots of the interaction effects of the moisture content andultrasonic treatment power on anthocyanin extraction; **C.** Response surface and contour plots of the effects of the moisture content and ultrasonic treatment time on anthocyanin extraction.

Determination of the optimal extraction process and validation of the regression model results

The optimal extraction process

On the basis of the established response surface model, an optimal parameter analysis was conducted to predict the ideal process conditions. The results indicated that the optimal conditions were as follows: ultrasonic treatment power of 165.98 W, ultrasonic treatment time of 19.57 minutes, and moisture content of 40.07%. Under these conditions, the anthocyanin extraction yield was approximately 42.5548 mg/g.

Validation of the regression model results

To ensure practical feasibility, the process conditions for anthocyanin extraction from *Aronia melanocarpa* were adjusted to an ultrasonic treatment power of 165 W, an ultrasonic treatment time of 20 minutes, and a moisture content of 40%. To validate the regression model, three replicate validation experiments were conducted using the optimized parameters. The measured extraction yield was approximately 42.54 ± 10.15 mg/g, which closely matched the predicted value, confirming the practical significance of the model.

Determination of purification conditions for anthocyanins from *Aronia melanocarpa*

Determination of the maximum absorption peak of the black fruit Aronia melanocarpa

Ultraviolet spectral scanning of the anthocyanin extract from *Aronia melanocarpa* was performed over the wavelength range of 150 to 800 nm. The smoothed results are presented in Fig. 3A.

As shown in Fig. 3A, absorption peaks are observed at wavelengths of 275, 370, and 540 nm. The change in the absorbance of anthocyanin is most pronounced at 540 nm, which falls within the visible light range. Considering that 275 nm lies in the ultraviolet region rather than the visible light region, and the fluctuation in the absorbance at 370 nm is relatively minor, 540 nm was ultimately selected as the optimal wavelength for determining the absorbance of anthocyanin.

Results of the static adsorption capacity of macroporous resins

The results of the static adsorption performance study on different stationary phase media are presented in Fig. 3B. As shown in the figure, all macroporous resins exhibit increased adsorption capacity with increasing time. Specifically, X-5 reaches adsorption equilibrium after one hour of treatment, with only minor fluctuations observed thereafter. AB-8 shows a similar trend to X-5 but exhibits a lower overall adsorption rate. In contrast, the adsorption capacity of polyacrylamide continues to increase steadily even after reaching equilibrium, although its adsorption rate remains relatively lower than that of the two resins. Overall, it is evident from the data that X-5 demonstrates superior adsorption performance relative to AB-8 and polyacrylamide.

Results of the static desorption capacity of macroporous resins

The results of the static desorption performance study on different stationary phase media are presented in Fig. 3C. As shown in the figure, polyacrylamide exhibits a high desorption rate, reaching up to 89.19% ± 0.19%. The desorption rate of X-5 is also relatively high, whereas that of AB-8 is significantly lower. On the basis of both the adsorption and desorption efficiencies, it can be concluded that polyacrylamide and X-5 demonstrate superior static treatment performance for the anthocyanins from *Aronia melanocarpa*.

Results of the dynamic elution test of macroporous resins

On the basis of the results from the preceding experiment, dynamic elution experiments were conducted on two resins: X-5 and polyacrylamide. The outcomes are presented in Fig. 3D.

The results of the dynamic elution experiments indicate that the absorbance of X-5 reaches its maximum at an elution volume of 10 mL. In contrast, polyacrylamide achieves its maximum absorbance at an elution volume of 15 mL. Moreover, the time required for complete elution is shorter for X-5 than for polyacrylamide, resulting in a more thorough elution process. Additionally, the elution of polyacrylamide exhibits a relatively high degree of variability. On the basis of these findings, we conclude that X-5 is the optimal stationary phase medium.

On the basis of a comprehensive analysis of the aforementioned results, both X-5 and polyacrylamide resins demonstrate favorable adsorption and desorption performance for the anthocyanins from *Aronia melanocarpa*. However, the stability of the adsorption and desorption process is notably superior with X-5. Therefore, we determined that X-5 macroporous resin is the optimal choice for the purification of anthocyanins from *Aronia melanocarpa*.

Selection results of the elution reagent

As shown in Fig. 3E, the desorption rate of anthocyanins significantly increases when the mobile phase concentration is within the range of 40% to 60%. Thereafter, the growth rate decelerates and stabilizes at a mass concentration of 80%, indicating that the optimal desorption efficiency is achieved at this point. When the concentration increased to 90%, no substantial improvement in the desorption rate was observed. Considering the cost-effectiveness of the selected solvent, an 80% ethanol solution by

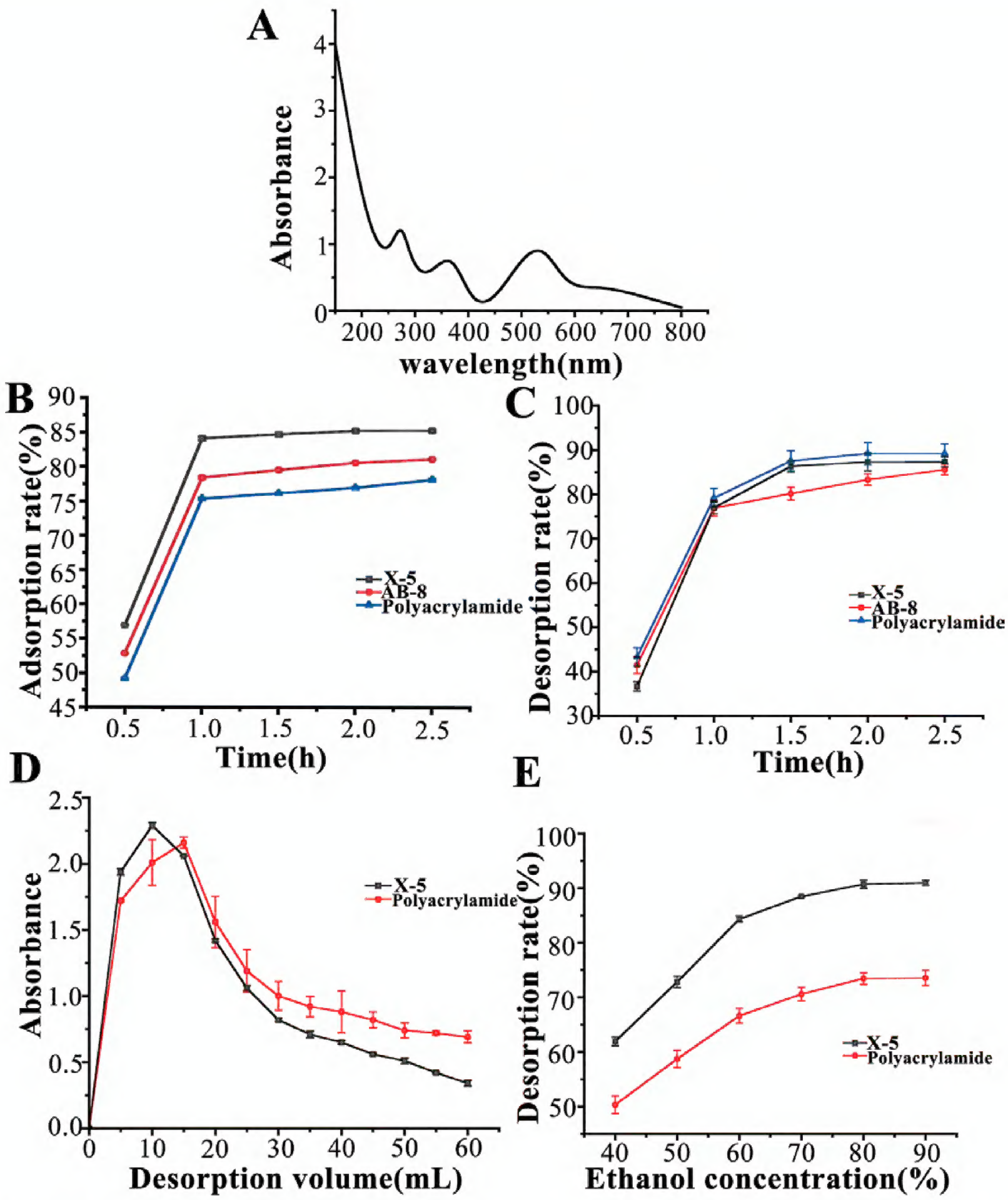

Figure 3. A. Absorbance of anthocyanin in *Aronia melanocarpa* at different wavelengths; **B.** Static adsorption curve of stationary phase medium; **C.** Static analysis of anthocyanins in stationary phase medium; **D.** Dynamic elution of anthocyanins in stationary phase medium; **E.** Effect of mobile phase concentration on different purification columns.

mass was ultimately chosen as the elution mobile phase for the purification process.

Discussion

The purification process employed in this experiment was insufficient and conducted on a small scale. Inadequate purification may lead to variations in the types and purity of anthocyanins in the final product, potentially affecting its nutritional value. With the widespread dissemination of the concept of a healthy diet, the application prospects of anthocyanins have increased. Liu et al. (2023) extracted anthocyanins from blueberries under three distinct conditions, offering a theoretical foundation for the preservation approaches of blueberries under various circumstances. Ge et al. (2022) identified and analyzed the types of anthocyanins in the residual leaves of different grape varieties. The results of their analysis revealed that the content of anthocyanins in red leaves was more than 20 times greater than that in the green leaves of the same grape variety, demonstrating that red leaves can serve as raw materials for the development of anthocyanin compounds and providing another significant basis for transforming agricultural waste into valuable resources. This study focused on the extraction and purification of anthocyanins from *Aronia*

melanocarpa. From the perspective of the extraction process, the ultrasonic treatment-assisted deep eutectic solvent (DES) method has favorable effects. The selected DES-2 (composed of glycerol and choline chloride) performs exceptionally well in extracting anthocyanins, with an extraction amount higher than that of traditional methods. This is attributed to the unique properties of DESs, such as low volatility and high solubility, providing an effective avenue for green extraction. The quadratic regression model, developed using a Box-Behnken experimental design, demonstrated highly significant results ($p < 0.0001$) with insignificant residual terms ($p = 0.8554$), indicating a robust fit for the model. The model exhibited high reliability and predictive accuracy, as evidenced by an R^2 value of 0.9968, an adjusted R^2 of 0.9928, and a signal-to-noise ratio of 39.84, exceeding the recommended threshold of 4. Analysis of variance (ANOVA) revealed the following order of interaction effects on anthocyanin extraction: ultrasonic power (A) > ultrasonic time (B) > moisture content (C), with ultrasonic power having the most significant impact (F-value = 572.095). Interaction analysis indicated marginal significance for the interactions between moisture content and ultrasonic power (AC) and between moisture content and ultrasonic time (BC), while no significant interaction was observed between ultrasonic power and ultrasonic time (AB). The optimized extraction conditions were found to be 165.98 W for ultrasonic power, 19.57 minutes for duration, and 40.07% moisture content, yielding a predicted anthocyanin extraction of 42.5548 mg/g. Experimental validation of these conditions (165 W, 20 min, 40% moisture) resulted in an actual extraction of 42.541 ± 0.15 mg/g, which closely matched the predicted value, with a relative error of only 0.03%, thereby fully validating the accuracy of the model. During the purification process, UV-Vis scanning identified 540 nm as the optimal absorption wavelength for anthocyanins. Comparative studies of three macroporous resins—X-5, AB-8, and polyacrylamide-revealed that X-5 resin exhibited superior performance in both static adsorption and dynamic elution. In static adsorption, X-5 resin achieved equilibrium within 1 hour with high desorption efficiency. In dynamic elution, X-5 reached peak absorbance at an elution volume of 10 mL, outperforming polyacrylamide. Based on these findings, X-5 resin was selected as the optimal purification medium, with 80% ethanol serving as the eluent, which maximized anthocyanin desorption stability. As a raw material rich in anthocyanins, *Aronia melanocarpa* has immense potential for development and utilization. Future research directions can be extended to explore more novel extraction and purification technologies to further increase the extraction rate and purity of anthocyanins and to conduct in-depth studies on the biological activity and functional characteristics of anthocyanins from *Aronia melanocarpa*, providing a more solid theoretical basis for their application in the fields of food, medicine, and cosmetics and promoting the advancement of the *Aronia melanocarpa* industry.

Conclusion

In this study, we conducted a comprehensive investigation into the extraction of anthocyanins from *Aronia melanocarpa* using ultrasonic treatment-assisted technology in conjunction with deep eutectic solvents. To achieve optimal extraction efficiency, response surface methodology (RSM) was employed to optimize key parameters in the extraction process. Through this approach, a robust regression model was developed that accurately predicted the extraction yield of anthocyanins under various conditions. On the basis of the model's predictions, we determined the optimal extraction conditions as 165W ultrasonic power, 20 minutes of ultrasonic exposure, and a 40% solvent moisture content. Under these conditions,, the anthocyanin extraction yield from black-fruit glandular rowan flowers was 42.541 ± 0.15 mg/g, which closely aligns with the model's predicted value, thereby validating the accuracy and reliability of the regression model. Additionally, the optimal conditions for purifying anthocyanins from *Aronia melanocarpa* were identified: an 80% ethanol solution as the elution mobile phase, X-5 macroporous resin as the adsorption material, and an optimal extraction wavelength of 540 nm.

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Competing interests

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Supplementary material

Supplementary material 1
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